Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray diffraction analysis of ybfF, a new esterase from *Escherichia coli* K12

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Received 24 September 2007
Accepted 2 November 2007

The product of the recently discovered *ybfF* gene, which belongs to the esterase family, does not show high sequence similarity to other esterases. To provide the molecular background to the enzymatic mechanism of the ybfF esterase, the ybfF protein from *Escherichia coli* K12 (Ec_ybfF) was cloned, expressed and purified. The Ec_ybfF protein was crystallized from 60% Tacsimate and 0.1 *M* bis-Tris propane buffer pH 7.0. Diffraction data were collected to 1.10 Å resolution using synchrotron radiation. The crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 66.09$, $b = 90.71$, $c = 92.88$ Å. With two Ec_ybfF molecules in the asymmetric unit, the crystal volume per unit protein weight is 2.17 Å^3 Da^{-1}, corresponding to a solvent content of 42%.

1. Introduction

Palmitoyl-CoA and malonyl-CoA are important cellular intermediates that are involved in the production of the storage lipid triacylglycerol, steroid compounds and fatty acids. Unbalancing the optimal cellular concentration of these molecules causes severe damage such as pseudoneonatal adrenoleukodystrophy and mild mental retardation in humans (Suzuki *et al.*, 2002; Brown *et al.*, 1984; Yano *et al.*, 1997). The turnover of palmitoyl-CoA and malonyl-CoA into their corresponding products is accomplished by thioesterases.

Esterases (EC 3.1.1.1) are enzymes that show a very broad substrate spectrum. They belong to the hydrolase family. Depending on substrate specificity, they are classified as esterases (EC 3.1.1) when they hydrolyze carboxylic esters, as thioesterases (EC 3.1.2) when thioester bonds are targeted and as phosphoesterases (EC 3.1.11–3.1.31) when phosphomonoester or phosphodiester bonds are their substrates. In *Escherichia coli*, for example, they are involved in diverse cellular processes such as lipid degradation and synthesis (Peist *et al.*, 1997), long-chain acyl-CoA hydrolysis (Li *et al.*, 2000), turnover of the antibiotic erythromycin (Ounissi & Courvalin, 1985; Arthur *et al.*, 1986), vitamin turnover (Sanishvili *et al.*, 2003), nucleic acid synthesis and degradation (Yakunin *et al.*, 2004; Sandigursky & Franklin, 1992) and so on.

The *ybfF* gene, which is conserved in various microorganisms, was annotated as a putative (thio)esterase (Blattner *et al.*, 1997) that contains an α/β-hydrolase fold upon analysis of its amino-acid sequence (Pfam; http://www.sanger.ac.uk/Software/Pfam). Like several lipases and esterases (Villena *et al.*, 2004; Zimmermann *et al.*, 2004), the ybfF esterase family possess a conserved G*X*S*X*G pentapeptide sequence that contains the nucleophilic serine residue. However, the ybfF proteins do not share high sequence similarity to other esterases (less than 20% sequence identity). Therefore, even their active-site environment has still not been revealed.

Ec_ybfF contains 254 amino acids and hydrolyzes the thioester or ester bonds of large substrates such as palmitoyl-CoA, malonyl-CoA and *p*-nitrophenyl butyrate (Kuznetsova *et al.*, 2005).

Even though a large number of structures of hydrolase-family members have been elucidated, the ybfF protein shows little sequence similarity to known hydrolase families. In order to understand the molecular mechanism of the ybfF protein at the atomic level, we have carried out the crystallization and preliminary X-ray crystallographic analysis of Ec_ybfF.

Table 1
Data-collection statistics for Ec_ybfF.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.23985
Space group	$P2_12_12_1$
Unit-cell parameters (Å, °)	a = 66.09, b = 90.71, c = 92.88, $\alpha = \beta = \gamma = 90$
Resolution range (Å)	50.0–1.10 (1.14–1.10)
Measured/unique reflections	1050868/212392
Completeness (%)	94.1 (88.5)
Mean $I/\sigma(I)$	11.9 (2.6)
$R_{\rm merge}$† (%)	3.4 (34.3)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \overline{I(hkl)}|/\sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity of an individual reflection and $\overline{I(hkl)}$ is the mean intensity of that reflection.

2. Methods

2.1. Cloning, expression and purification of ybfF

The *E. coli* gene coding for ybfF (residues 1–254) was amplified from *E. coli* chromosomal DNA by the polymerase chain reaction (PCR). The PCR product was then cloned into pET21a (Invitrogen). The expression construct was transformed into *E. coli* B834(DE3) and grown in LB medium containing 100 µg ml^{-1} ampicillin at 310 K. After induction with 1.0 m*M* IPTG for a further 8 h at 310 K, the culture was harvested by centrifugation at 5000*g* at 277 K. The cell pellet was resuspended in ice-cold buffer *A* (20 m*M* Tris–HCl pH 8.0 and 500 m*M* NaCl) and disrupted by ultrasonication. The cell debris was removed by centrifugation at 11 000*g* for 1 h. The Ec_ybfF fusion protein was purified using a 5 ml HisTrap chelating column (GE Healthcare, Uppsala, Sweden) and the bound protein was eluted with a linear gradient from 0 to 500 m*M* imidazole in buffer *A*. After removal of salts, the protein was purified using a 5 ml HiTrapQ anion-exchange column (GE Healthcare, Uppsala, Sweden). For further purification, size-exclusion chromatography was performed using Sephacryl S-300 HR (GE Healthcare, Uppsala, Sweden) in a buffer consisting of 20 m*M* Tris–HCl pH 8.0 and 200 m*M* NaCl. The purified protein was >95% pure as judged by Coomassie Blue-stained SDS–PAGE. The purified protein has eight extra amino acids LEHH-HHHH at the C-terminus.

2.2. Crystallization

For crystallization, the purified Ec_ybfF protein was concentrated to 25 mg ml^{-1} in a buffer consisting of 20 m*M* Tris–HCl pH 7.5 and 300 m*M* NaCl. The initial crystallization conditions for Ec_ybfF were obtained using sparse-matrix screens (Jancarik & Kim, 1991) from Hampton Research and Emerald BioSystems. Crystals suitable for diffraction experiments were obtained within 2 d by mixing 1 µl sample solution and 1 µl reservoir solution consisting of 60%(*w*/*v*) Tacsimate (Hampton Research) and 0.1 *M* bis-Tris propane pH 7.0 at 291 K (Fig. 1).

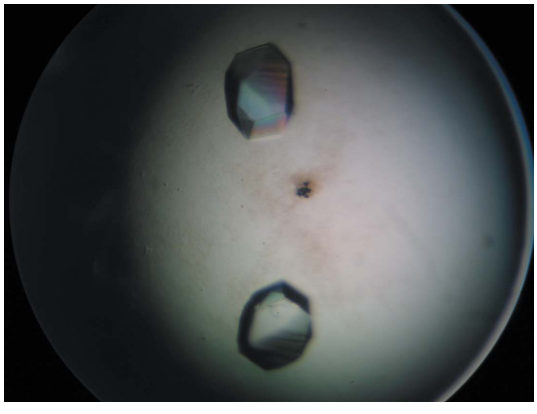

Figure 1
Crystals of Ec_ybfF. The crystals grew within 2 d at 291 K to maximum dimensions of approximately 0.4 × 0.4 × 0.4 mm.

2.3. Data collection

For data collection, crystals were briefly immersed in precipitant solution containing 10%(*v*/*v*) glycerol as a cryoprotectant and immediately placed in a 100 K nitrogen-gas stream. Native X-ray diffraction data were collected at MAXII6C, Pohang Accelerator Laboratory (PAL, Korea). The crystal was exposed for 1 s per 1° oscillation. 180 images were collected with a crystal-to-detector distance of 65 mm. The crystal diffracted to 1.1 Å resolution (Fig. 2). The data were indexed and scaled with *HKL*-2000 (Otwinowski & Minor, 1997). Data-collection statistics are summarized in Table 1.

3. Results and discussion

We have successfully expressed the Ec_ybfF protein using a bacterial expression system and purified it. The crystals obtained diffracted to 1.10 Å resolution and belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 66.09, b = 90.71, c = 92.88 Å, $\alpha = \beta = \gamma = 90°$. The asymmetric unit contains two Ec_ybfF molecules as judged by self-rotation function analysis, with a corresponding crystal volume per protein weight of 2.17 Å^3 Da^{-1} (including the extra amino acids added during the cloning step) and a solvent content of 42% (Matthews, 1968).

We have attempted molecular-replacement methods for phase determination using various hydrolase folds, but have not yet been successful. Therefore, the crystal structure of Ec_ybfF is now being

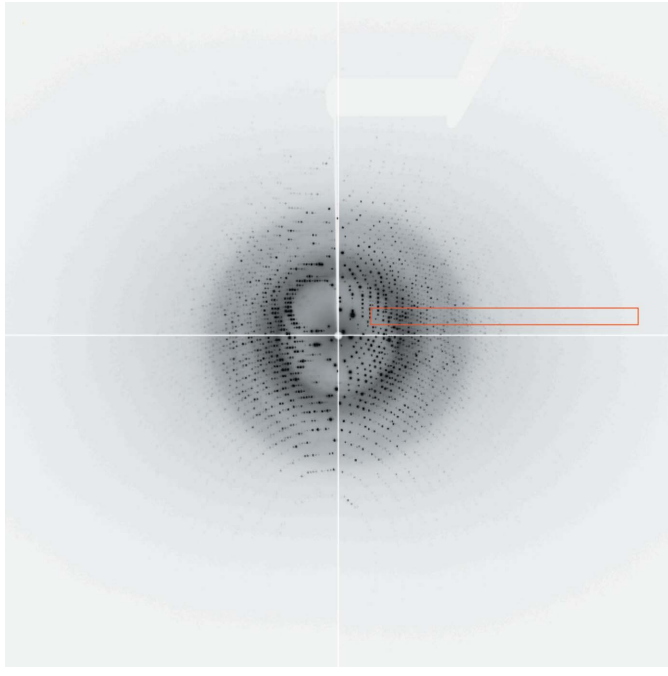

Figure 2
Representative X-ray diffraction image from Ec_ybfF. The crystal was exposed for 1 s over a 1° oscillation range. The edge of the detector corresponds to a resolution of 1.00 Å.

solved by the MAD or SAD method with selenium as the anomalous scatterer using synchrotron radiation.

This work was supported by a CNU Specialization Grant funded by Chonnam National University and by CNU Special Grant for Administration No. 2006-0146. We thank Dr K. J. Kim, Pohang Light Source for help with data collection.

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